



Beauty is in the eye of the beholder: Cruciform eye reveals new species of direct-developing frog (Strabomantidae, *Pristimantis*) in the Amazonian Andes

Germán Chávez^{1,2}, Luis A. García-Ayachi^{1,2}, Alessandro Catenazzi^{1,2,3}

¹ Instituto Peruano de Herpetología (IPH), Augusto Salazar Bondy 136, Urb. Higuiereta, Surco, Lima, Perú

² División de Herpetología, Centro de Ornitología y Biodiversidad (CORBIDI), Santa Rita No. 105 Of. 202, Urb. Huertos de San Antonio, Surco, Lima, Perú

³ Department of Biological Sciences, Florida International University, 11200 SW 8th St., Miami, FL 33199, USA

<http://zoobank.org/117AA0B4-99A2-4F4F-85A9-B9FAE8E47BB1>

Corresponding author: Germán Chávez (vampflack@yahoo.com)

Academic editor: Martin Husemann ♦ Received 27 January 2021 ♦ Accepted 22 March 2021 ♦ Published 9 April 2021

Abstract

We describe a new species of frog from the eastern slopes of the Andes in central Peru. *Pristimantis sira* **sp. nov.** has a distinctive crossing mark on the iris and no tympanum. The new species is closely related to *P. antisuyu* Catenazzi & Lehr, 2018, *P. cruciocularis* Lehr, Lundberg, Aguilar & von May, 2006, and *P. erythroinguinis* Catenazzi & Lehr, 2018, but is easily differentiable by lacking colour blotches on groins. *Pristimantis sira* **sp. nov.** inhabits the mountain forests from 1550 to 2200 m a.s.l., inside a national reserve threatened by illegal mining.

Key Words

Andes, crossing mark, iris, national reserve, illegal mining

Introduction

Expeditions to remote places in the eastern slopes of the Peruvian Andes have shown that unexplored neotropical mountains are sources of unknown and endemic herpetofauna (Duellman et al. 2014; Catenazzi et al. 2015; Echevarría et al. 2015; Chávez and Catenazzi 2016; Chávez et al. 2017; Lehr and Moravec 2017; Lehr and von May 2017; Lehr et al. 2017a, 2017b). Frogs are one of the most diverse herpetofaunal orders, particularly in Andean ecosystems where endemism is high (Swenson et al. 2012). Furthermore, many of the discoveries of new amphibians are frogs (Duellman et al. 2014; Catenazzi et al. 2015; Chávez and Catenazzi 2016; Lehr and Moravec 2017; Lehr and von May 2017; Lehr et al. 2017a, 2017b). Among recently named frogs, most of the new Peruvian Andean species belong to the genus *Pristimantis* Jiménez

de la Espada, 1870, which is the most diverse clade in the Neotropics (Padial et al. 2014). Over the last ten years, herpetologists have named seven new species of *Pristimantis* from the eastern Andes of central Peru (Shepack et al. 2016; Lehr and Moravec 2017; Lehr and von May 2017; Lehr et al. 2017a, b), showing that these mountains host a still unknown anuran diversity, and that *Pristimantis* are one of the most diverse clades of these amphibian communities.

El Sira Communal Reserve is located on the eastern slopes of the Andes in central Peru, and protects about 616413 hectares of primary forest. El Sira is the highest cordillera adjacent to the Ucayali River, going from 200 m to 2200 m a.s.l., and is bordered by the Pachitea river (which eventually flows into the Ucayali), further isolating the El Sira mountains as the eastern branch of the Andes in the Ucayali basin (see Figure 5). Because

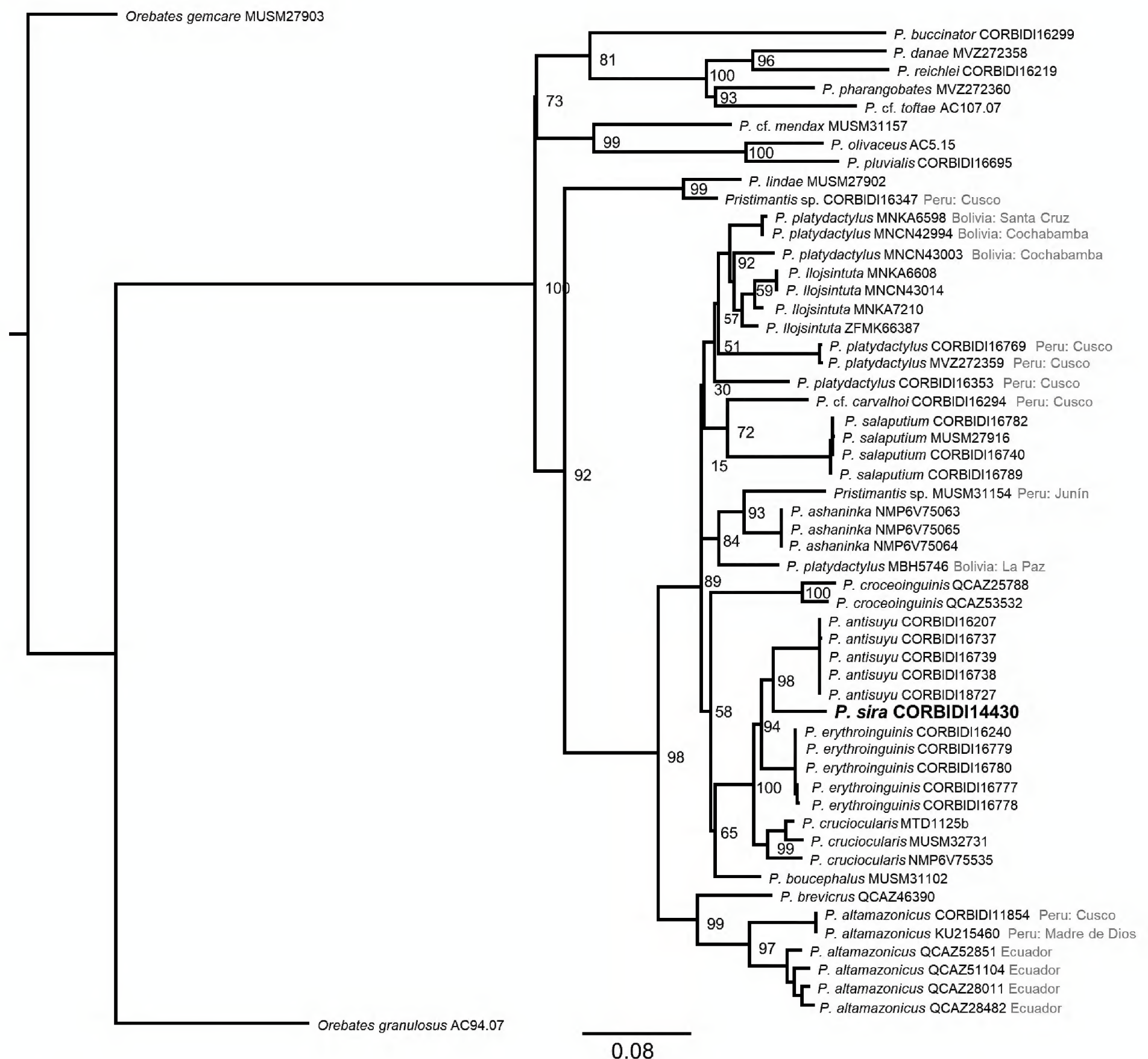


Figure 1. Phylogenetic analysis based on concatenated dataset of 16S, CO1 and RAG sequences showing the relationship between the new species (highlighted in bold) and its congeners. ML bootstrap values are indicated at each node.

of the hard access and rough topography, El Sira is likely one of the most unexplored places of Peru. Only a handful of herpetological expeditions (Duellman and Toft 1979; Aichinger 1991; Whitworth et al. 2016a, 2016b) have reached these forests, resulting in the description of four anuran species (Duellman and Toft 1979; Aichinger 1991; Lötters and Henzl 2000), all of them endemic from El Sira.

Nevertheless, none of the four endemic frogs described so far is a *Pristimantis*, in contrast to other mountain chains in Peru where most of the recently described species belong to this genus (Shepack et al. 2016; Lehr and Moravec 2017; Lehr and von May 2017; Lehr et al. 2017a, 2017b). The systematics of *Pristimantis* is challenging because it often requires an integrative taxonomy approach for species delimitation, and because genetic sequences are not available for many species.

During fieldwork we performed in El Sira Communal Reserve from 2013 to 2014, we collected a series of unidentified *Pristimantis* frogs. Genetic and morphological analysis revealed that these specimens belong to an unnamed species, which we describe below.

Material and methods

We follow Lynch and Duellman (1997) for the format of diagnosis and description of the new species. For systematics of Strabomantidae we follow Hedges et al. (2008), Blackburn and Wake (2011) and Padial et al. (2014). We collected specimens during the night while conducting Visual Encounter Surveys (Crump and Scott Jr 1994). We euthanized specimens with an 8% benzocaine solution, fixed them in 10% formalin, and stored

them in 70% ethanol. We deposited all specimens in the herpetological collection of Centro de Ornitología y Biodiversidad (CORBIDI). The Servicio Nacional de Areas Protegidas de Peru issued collecting permit RJ N° 003-2014-SERNANP-RCS-JEF029-2016-SERFOR-DGGSPFFS. We measured the following variables to the nearest 0.1 mm with digital callipers under a stereoscope, as described in Duellman and Lehr (2009): snout–vent length (SVL); eye–nostril distance (E–N); head length (HL); head width (HW); interorbital distance (IOD); internarial distance (IND); tibia length (TL); foot length (FL); eye diameter (ED); upper eyelid width (EW). Fingers and toes are numbered preaxially to postaxially from I–IV and I–V respectively. We determined comparative lengths of toes III and V by adpressing both toes against Toe IV; lengths of fingers I and II were determined by adpressing the fingers against each other. Specimens were sexed based on external sexual characteristics (e.g., presence of vocal sacs in males), and internal dissection of the gonads. Photographs were taken in the field by GC, and in the laboratory by LAGA. We used these photos for descriptions of coloration in life and in preserved condition, respectively. In addition to the type series of the new species, we examined specimens of related congeners (Suppl. material 1) or obtained morphological data from the original description for diagnostic comparisons.

We performed phylogenetic analyses on a concatenated dataset of fragments of two mitochondrial genes (16S rRNA and cytochrome oxidase I, COI) and one nuclear gene (RAG1) to examine relationships with the hypothesized closest relatives *P. antisuyu* Catenazzi & Lehr, 2018, *P. cruciocularis* Lehr, Lundberg, Aguilar & von May, 2006, and *P. erythroinguinis* Catenazzi & Lehr, 2018, as well as related species of the *P. llojsintuta* Köhler & Lötters, 1999 – *P. platydactylus* Boulenger, 1903 complex. We extracted DNA from tissues of the holotype, CORBIDI 14430 by using a commercial extraction kit (IBI Scientific, Peosta, USA). We followed standard protocols for amplification and sequencing of DNA (Hedges et al. 2008; Catenazzi and Tito 2016). We used the 16S_r forward (5′–3′ sequence: CGCCTGTTTATCAAAACAT) and 16S_r reverse (5′–3′ sequence: CCGGTCTGAACCTCAGATCACGT) primers for 16S, the Chm4 forward (TYTCWACWAAYCAYAAAGAYATCGG) and Chm4 reverse (ACYTCRGGRTGRCCRAARAATCA) primers for COI, and the R182 forward (GCCATAACTGCTGGAGCATYAT) and R270 reverse (AGYAGATGTTGCCTGGGTCTTC) primers for RAG1 (Palumbi et al. 2002; von May et al. 2017). We used the following thermocycling conditions during the polymerase chain reaction (PCR) with a ProFlex thermal cycler (Applied Biosystems): one cycle of 96 °C/3 min; 35 cycles of 95 °C/30 s, 55 °C/45 s, 72 °C/1.5 min; one cycle 72 °C/7 min. We purified PCR products with Exosap-IT (Affymetrix, Santa Clara, CA) and shipped the purified products to MCLAB (San Francisco, CA) for sequencing. Newly generated sequences were deposited in GenBank (Suppl. material 2).

We downloaded sequences of closely related (on the basis of BLAST results for 16S) or morphologically similar, congeneric species and of two species of *Oreobates* (used as outgroup taxa) from GenBank (Suppl. material 2). We used Geneious R11, version 11.1.5 (Biomatters, <http://www.geneious.com/>) to assemble pair-end reads, to generate a consensus sequence, to align our novel and GenBank sequences with the alignment program MAFFT v7.017 (Katoh and Standley 2013), and also to concatenate sequences of the three genes. We trimmed aligned sequences to a length of 571 bp for 16S, 678 for COI, and 645 bp for RAG1 (total length 1894 bp), after removing regions of ambiguous alignment for 16S. Our analysis included 56 terminals. We used PartitionFinder, v. 1.1.1 to select the best partitioning scheme and substitution model for each gene using the Bayesian information criterion (BIC). The best partitioning scheme included six partitions: subset 1 for 16S with the model GTR + I + G, subset 2 for the first codon position of COI with SYM+I+G, subset 3 for the second codon position of COI with TVM+I, subset 4 for the third codon position of COI with TIM+G, subset 5 for the first and second codon positions of RAG1 with HKY+G, and subset 6 with the third codon positions of RAG1 with K81+G.

We inferred phylogenetic relationships with Maximum Likelihood (ML) inference. We conducted the analysis with IQ-TREE v1.6.12 (Nguyen et al. 2015) using our concatenated dataset, the partitioning and substitution models determined by PartitionFinder, and the ultrafast bootstrap method (10000 bootstrap alignments). We also estimated genetic distances for the 16S rRNA mitochondrial fragment to provide further support of species delimitation. Although there is not a set threshold for delimiting species, Fouquet et al. (2007) suggested that 3% distance for 16S is a reasonable criterion to identify putative new species. The benefit of using 16S is that this fragment is the most frequently sequenced marker for anuran taxonomy (Fouquet et al. 2007; Padial et al. 2009; Vences et al. 2005), including for species of Holoadeninae (Hedges, Duellman & Heinicke, 2008). We estimated uncorrected p-distances (i.e., the proportion of nucleotide sites at which any two sequences are different) with the R package “ape” (Paradis et al. 2004), and uploaded the table to Figshare (<https://doi.org/10.6084/m9.figshare.13640615>).

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) and the associated information can be viewed through any standard web browser at <http://zoobank.org/117AA0B4-99A2-4F4F-85A9-B9FAE8E47BB1>.

Results

Generic placement

Our study (Figure 1) supports the generic placement of the new species and its distinctiveness with respect to similar species. According to phylogenetic analyses, the closest related species are *P. antisuyu* Catenazzi and Lehr 2018, *P. cruciocularis* Lehr, Lundberg, Aguilar, von May 2006, and *P. erythroinguinis* Catenazzi and Lehr 2018. These four species form a well-supported clade of mostly Andean foothill and montane forest small *Pristimantis* with cruciform eyes and, except for *P. sira* sp. nov., yellow or red coloration on groin and belly. The close relationships among *P. antisuyu*, *P. cruciocularis*, *P. erythroinguinis* and *P. sira* sp. nov. are supported by 16S rRNA genetic distances (Figshare <https://doi.org/10.6084/m9.figshare.13640615>), which are smallest for pairwise comparisons among these four species (range 7.2–10.2%) than they are for comparisons with other species of *Pristimantis* (all distances >10.2%).

Description

Pristimantis sira sp. nov.

<http://zoobank.org/6579CBED-1CF0-4D59-8B37-FDB405EFB352>

Figs 1–3, 4D

Holotype. PERU • 1 ♀, adult; Huánuco department, Huánuco province, Campamento Peligroso, El Sira Communal reserve; 9°25'34.2"S, 74°44'6.6"W; 1520 m a.s.l.; 24 March 2014; G. Chávez and Jose Malqui; CORBIDI 14430.

Paratopotypes. PERU • 1 ♂, 2 ♀, adults and a juvenile; same data as for holotype; 01 December 2013; G. Chávez; CORBIDI 14433 (Figure 3C, D), 14429 (Figure 3E, F), 14432, 13952.

Paratypes. PERU • 1 ♀, adult and a juvenile; Huánuco department, Pachitea province, Campamento La Cumbre; 9°25'27.0"S, 74°42'47.0"W; 2145 m a.s.l.; 30 November 2013; G. Chávez; CORBIDI 13933, 13944.

Diagnosis. The new species is diagnosed by the following combination of characters: (1) skin on dorsum finely shagreen with a few scattered subconical tubercles, that on venter areolate, W-shaped scapular fold present, discoidal fold absent, dorsolateral folds absent; (2) tympanic membrane and tympanic annulus absent, supratympanic fold absent; (3) snout acutely rounded from dorsal view, moderate in length and rounded from lateral view, canthus rostralis weakly concave in dorsal view, angular in lateral view, loreal region concave, rostral papilla or keel absent; (4) upper eyelid bearing two or three sub conical small tubercles, narrower than IOD, cranial crests absent; (5) dentigerous process of vomers absent; (6) males with vocal sacs and vocal slits, nuptial excrescences absent; (7) heels lacking tubercles; (8) finger I shorter than finger II, tips of digits expanded, bearing circumferential

grooves, discs about 1.5 times wider than digits in fingers I, II and III, finger IV bearing a rounded disc about twice as wide as its digit; (9) fingers with narrow lateral fringes; (10) antebrachial tubercle absent; (11) ulnar and tarsal tubercles absent; (12) inner metatarsal tubercle oval twice as long as round outer metatarsal tubercle, low supernumerary plantar tubercles at the base of toes II, III, IV and V; (13) toes without lateral fringes, webbing absent, toe V longer than toe III; (14) in life, dorsum yellowish-brown, dark brown or olive brown with dark transversal bands; interorbital bar dark brown; canthus rostralis paler than loreal region and dark bordered; dark labial bars present; throat, chest, and belly dark brown or dark grey with scattered white flecks; groins, posterior surfaces of thighs, and shanks dark brown; iris copper yellow with a vertical black line and dark reticulations, black pupil surrounded by a copper orange ring; (15) SVL 12.9–14.7 mm in males; 19.0–20.4 mm in females.

Comparisons. The combination of having a vertical line crossing the iris (which forms a cruciform mark) and lacking tympanic annulus and membrane distinguishes *Pristimantis sira* sp. nov. from most congeners except *P. altamazonicus* Barbour & Dunn, 1921, *P. antisuyu*, *P. ashaninka* Lehr Moravec, 2017, *P. cruciocularis* and *P. erythroinguinis*. However, *P. sira* is easily differentiable from all of them by (condition for the other species in parenthesis): having 2–3 small sub conical tubercles on upper eyelid (vs a single conical tubercle in *P. altamazonicus*, 4–6 small tubercles in *P. antisuyu*, enlarged conical tubercles in *P. ashaninka*, 2–5 small tubercles in *P. cruciocularis*, and 4–6 large and small tubercles in *P. erythroinguinis*), groins and posterior surface of thighs dark without spots or flecks (vs bearing red or yellow blotches in *P. cruciocularis*, yellow blotches in *P. antisuyu*, red blotches in *P. erythroinguinis*, see Figure 4), vocal slits in males (vs absent in *P. altamazonicus*, *P. antisuyu*, *P. cruciocularis* and *P. erythroinguinis*), vomerine teeth absent (vs present in *P. altamazonicus*, *P. antisuyu*, *P. ashaninka*, *P. cruciocularis* and *P. erythroinguinis*), and heels lacking tubercles (vs present *P. altamazonicus*, *P. antisuyu*, *P. ashaninka*, *P. cruciocularis* and *P. erythroinguinis*). Additionally, *P. sira* is genetically related to *P. croceinguinis* Lynch, 1968, and *P. boucephalus* Lehr, Moravec, Cusi, Gvozdk, 2017. Nevertheless, *P. sira* is distinct in bearing dark groins without blotches (vs yellow groin in *P. croceinguinis* and *P. boucephalus*), skin on dorsum shagreen (vs tuberculate in *P. croceinguinis* and smooth in *P. boucephalus*), upper eyelid having two or three sub conical tubercles (vs tubercles absent in *P. croceinguinis*), lacking dentigerous process of vomers (vs present in *P. croceinguinis*), and heels lacking tubercles (vs conical tubercle on heels present in *P. boucephalus*).

Description of the holotype. An adult female (CORBIDI 14430; Figure 2A–E, 3A, B) with a SVL of 19.6 mm, head as wide as long (Fig. 2A, B); snout acutely rounded in dorsal view and rounded in lateral view, short (eyenostril distance 9.8% of SVL); canthus rostralis distinct in lateral view; loreal region concave; nostrils protuberant,

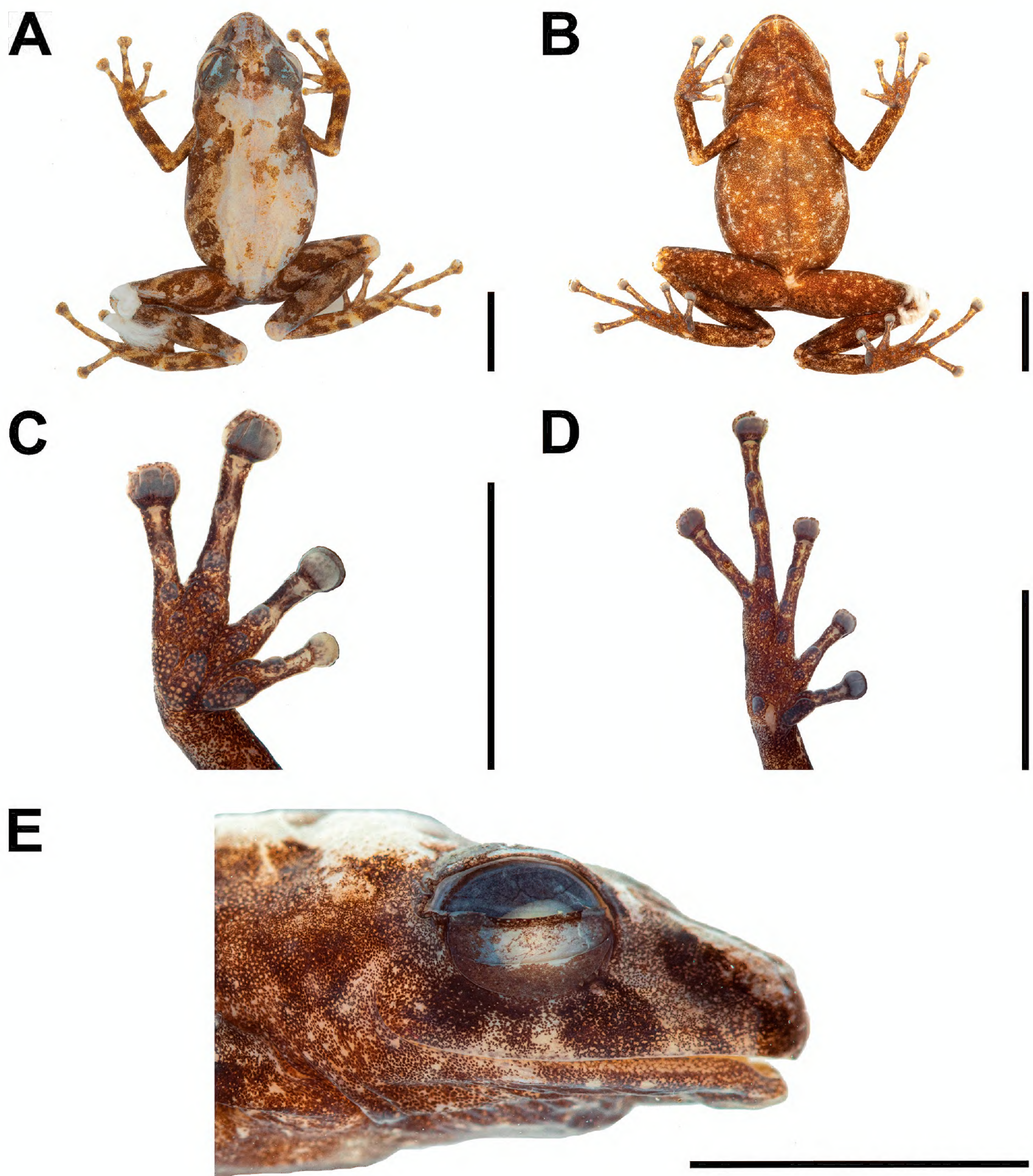


Figure 2. Holotype of *Pristimantis sira* sp. nov. in preservative (CORBIDI 14430). (A) dorsal view of the body; (B) ventral view of the body; (C) ventral view of the right hand; (D) ventral view of the right foot; (E) lateral view of the head. Photographs by Luis A. García-Ayachi. Scale bar = 5 mm.

directed anteriorly; interorbital area flat, slightly broader than upper eyelid (upper eyelid width 89% of interorbital distance); cranial crests absent; upper eyelid bearing two small subconical tubercles; tympanic membrane absent; tympanic annulus absent (Fig. 2E); postrictal tubercles absent. Choanae small, rounded, not concealed by palatal shelf of maxillary; tongue longer than wide and granular, dentigerous processes of vomers absent. Skin texture

on dorsum and flanks finely shagreen; dorsolateral folds absent; venter areolate; thoracic fold present, discoidal fold absent, cloacal sheath absent. Forearm slender; ulnar tubercles absent, ulnar fold absent; radio-ulnar length 22% of SVL; fingers with narrow lateral fringes; relative lengths of fingers $I \leq II < IV < III$; palmar tubercle bilobed, thenar tubercle oval (Fig. 2C); subarticular tubercles round, prominent; supernumerary palmar tubercles

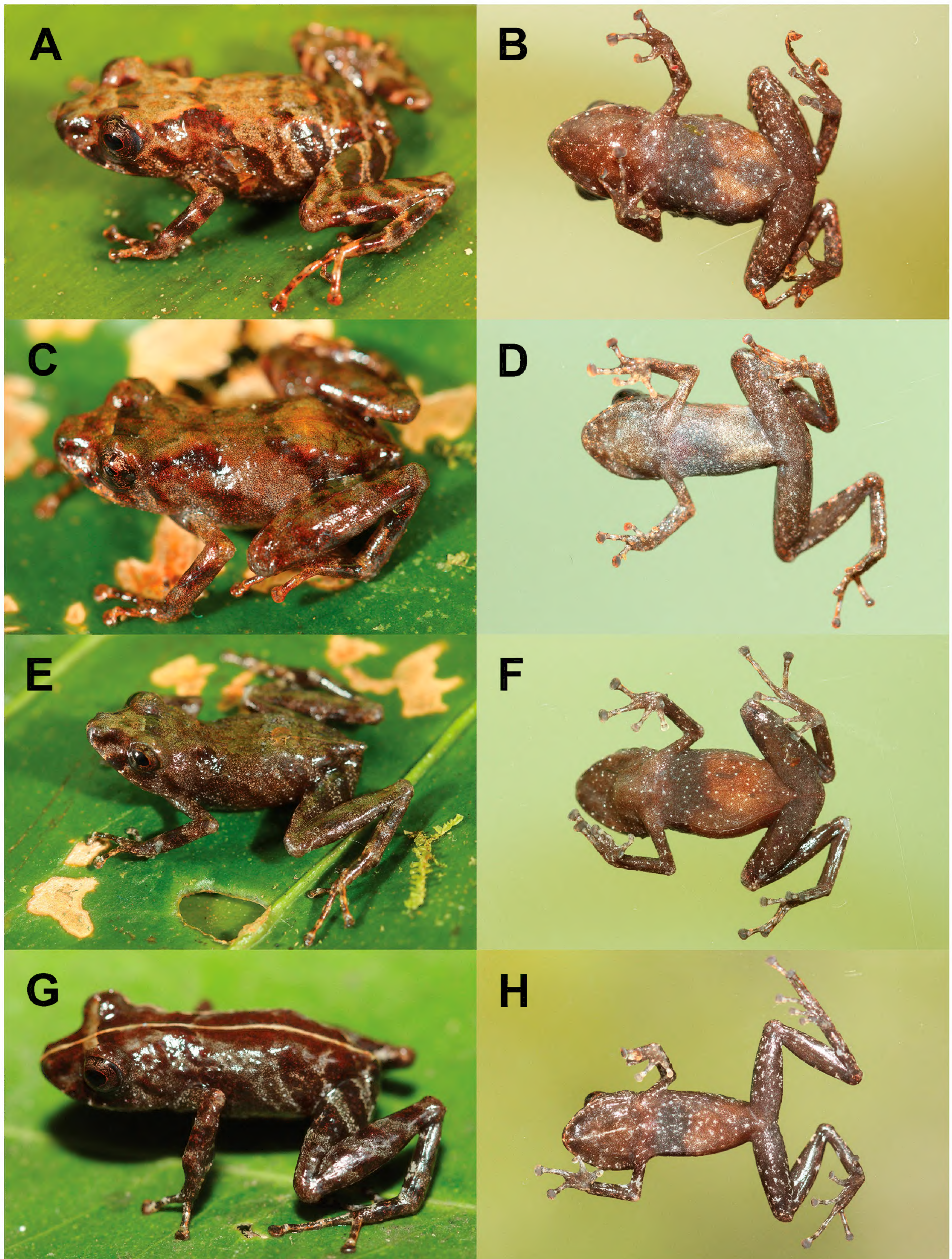


Figure 3. Coloration in life of the type series of *Pristimantis sira* sp. nov.: (A, B) adult female (CORBIDI 14430, holotype); (C, D) adult male (CORBIDI 14433); (E, F) adult female (CORBIDI 14429); (G, H) juvenile male (CORBIDI 13952). Photographs by Germán Chávez.

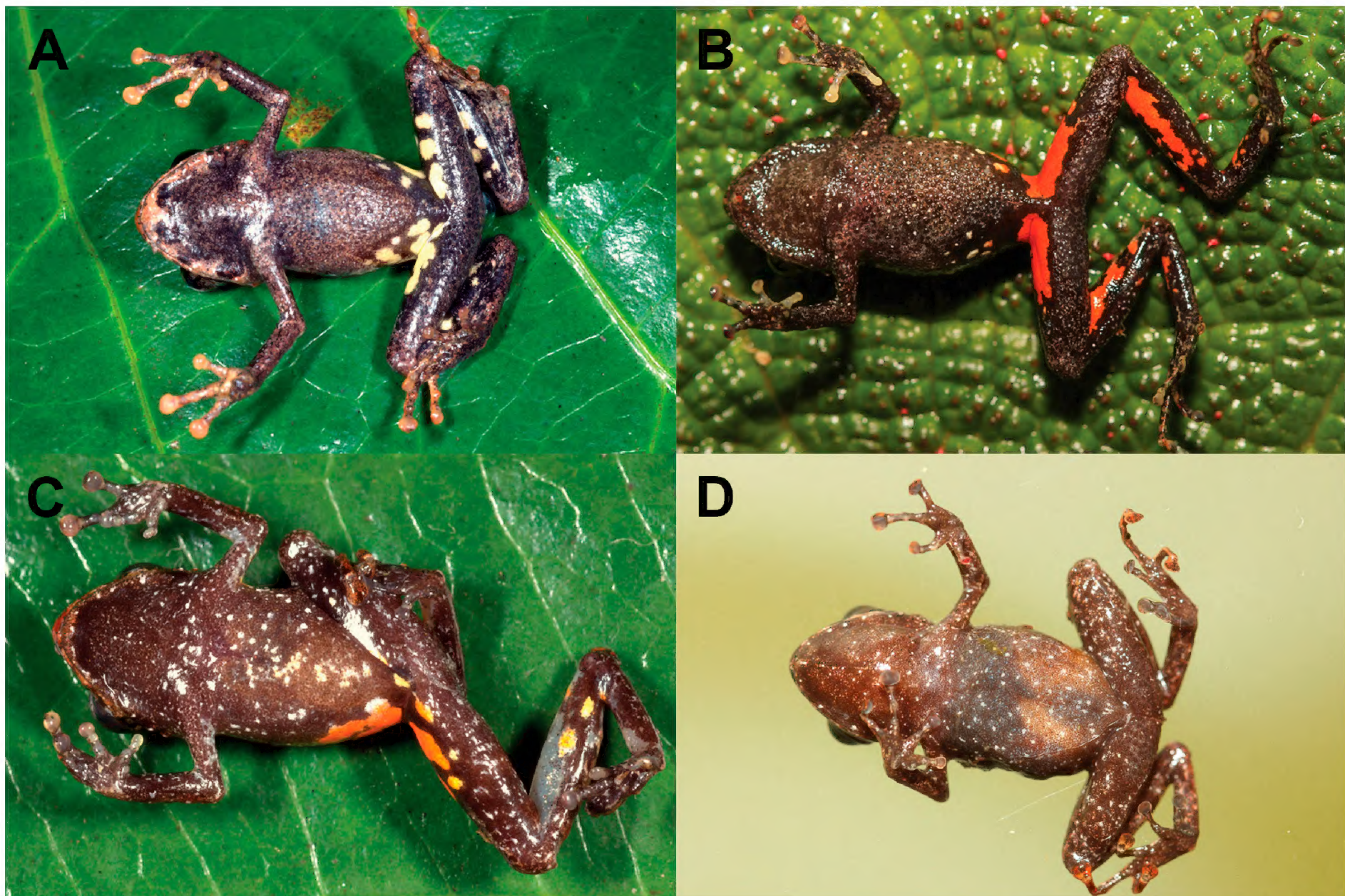


Figure 4. Ventral view in life of *Pristimantis cruciocularis* group's species: (A) *P. antisuyu* (CORBIDI 18726); (B) *P. cruciocularis* (CORBIDI 11554); (C) *P. erythroinguinis* (MUSM 30468); (D) *P. sira* sp. nov. (CORBIDI 14430). Photographs by Alessandro Catenazzi (A, C) and Germán Chávez (B, D).

present at the base of all fingers; disc cover finger I barely expanded, those of fingers III and IV extensively expanded (Fig. 2C), outer discs of fingers as wide as those of toes; discs covered with elliptical ventral pads defined by circummarginal grooves. Hind limbs slender; tibia length 48% of SVL; foot length 94% of tibia length; tarsal fold absent, tarsal tubercles absent; heel lacking tubercles; toes without lateral fringes; subarticular tubercles round, prominent; inner metatarsal tubercle oval, about 2.4 times the size of subconical outer tubercle (Fig. 2D); supernumerary plantar tubercles low at the base of all toes; discs covers slightly expanded; toes with defined pads; discs pads nearly elliptical; relative length of toes $I < II < III < V < IV$; tip of toe V reaching proximal border of distal subarticular tubercle IV; tip of toe II reaching proximal border of distal subarticular tubercle of Toe III (Fig. 2D).

Measurements (in mm) and proportions of the holotype: SVL = 19.6; HL = 6.7; HW = 7.2; ED = 2.7; E-N = 1.9; IOD = 1.8; EW = 1.6; IND = 1.7; TL = 9.5; FL = 9.0; HL/SVL = 0.3; HW/SVL = 0.3; EW/IOD = 0.8; E-N/ED = 0.7; TL/SVL = 0.4; FL/SVL = 0.4; FL/TL = 0.9.

Coloration in life (Fig. 3A, B). Dorsum, flanks, and dorsal surface of limbs are yellowish-brown with dark brown transversal bands and reddish-orange tubercles; dorsal surface of head bearing a dark brown interorbital bar; canthus rostralis yellowish-brown, loreal region dark

brown, labial bars dark brown, and white minute flecks present on the upper labial region. Throat, chest, belly, ventral surface of limbs, hands and feet dark brown with white flecks; groins, posterior surface of thighs, and posterior surface of shanks dark brown. Anterior surface of thighs is dark brown. Iris copper yellow with a vertical black line and dark reticulations, black pupil surrounded by a copper orange ring.

Coloration in preservative (Fig. 2A, B). Despite the skin on dorsum suffering damage, the coloration is similar to coloration in life, except that dark brown coloration turned yellowish-brown. The venter, limbs and ventral surfaces of hands and feet are yellowish-brown with creamy-white flecks, discs on hands and feet dark grey; iris gray.

Intraspecific variation. Dorsal coloration from yellowish brown to dark brown in juvenile CORBIDI 13952 (Figure 3G, H) and olive brown in female CORBIDI 14429 (Figure 3E, F). Moreover, CORBIDI 13952 has a pattern consisting of a middorsal creamy white line going from the tip of the nose to the cloaca, a pale interorbital bar, and a thin creamy white mid-throat line going from the chin to the distal edge of the throat. Furthermore, male CORBIDI 14433 (Figure 3C, D) has a yellowish-brown throat, a greyish-white belly, and yellowish-brown coloration on the ventral and posterior surface of thighs. Table 1 reports variation in measurements and proportions.

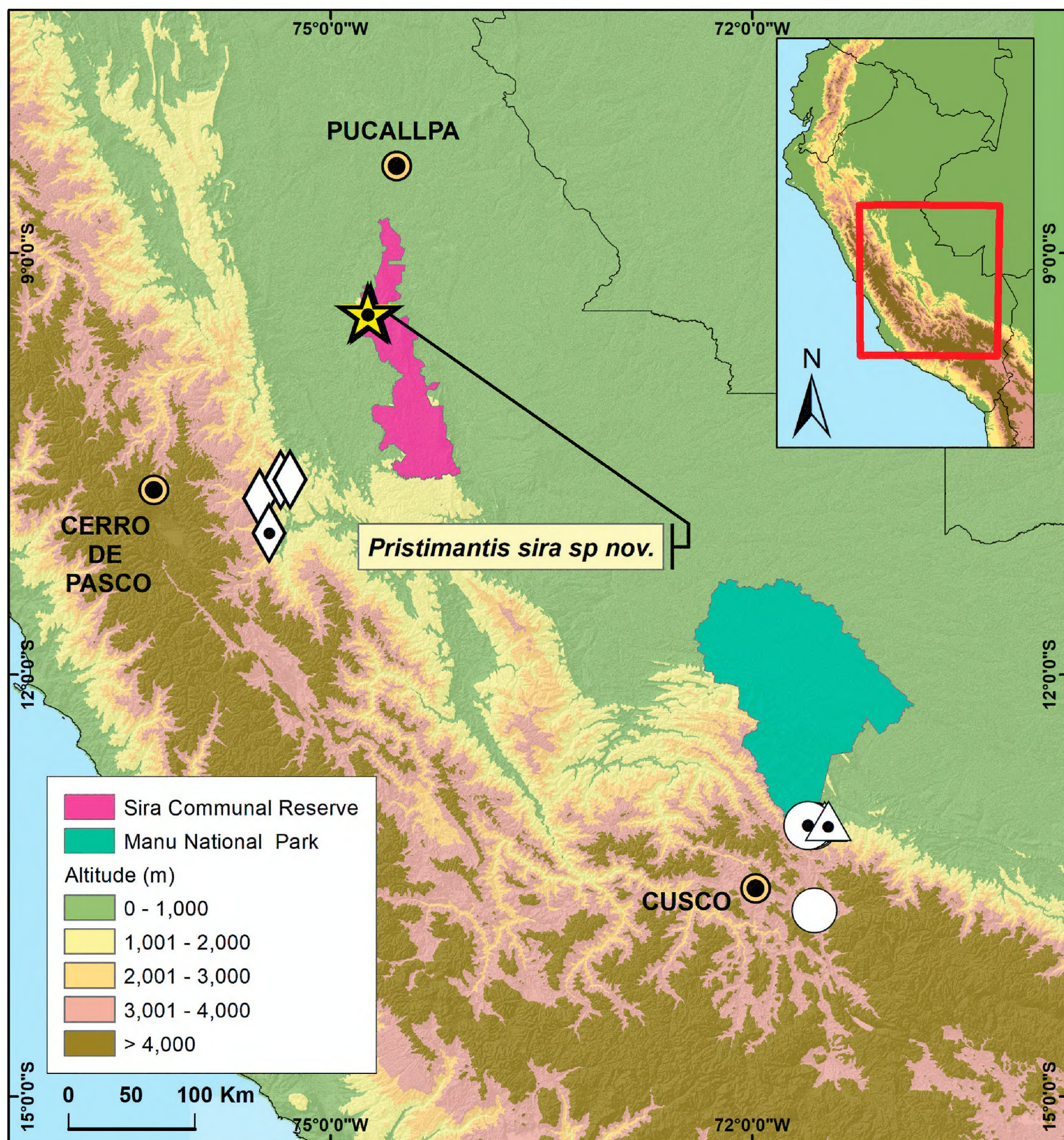


Figure 5. Map indicating the type locality of *Pristimantis sira* sp. nov. (yellow star), as well as type localities (symbols with a central black point) and distribution of the species of the *P. cruciocularis* group: *P. antisuyu* (white circles), *P. cruciocularis* (white rhomboids), *P. erythroinguinis* (white triangles).

Etymology. The species epithet “sira” is a noun in apposition, referencing El Sira Communal Reserve, a protected area established in 2001, containing the type locality of the new species. El Sira also protects one of the last large extensions of primary mountain forests in Central Peru.

Distribution, natural history and threats. We observed *Pristimantis sira* sp. nov. on leaves, at 1–1.7 m height, in the forests of the eastern slopes of the mountains of El Sira Communal Reserve, between 1500–

2200 m a.s.l (Figure 5). The habitat at 1500 m a.s.l. is a montane forest, with riparian vegetation consisting mainly of bushes, tree ferns and trees canopy ~20 m tall (Figure 6A, C). Climbers are also present (lianas) as well as low epiphytes, ferns, mushrooms, and lichens on the ground. Low trees (no more than 8–10 m tall) dominate the forest at 2000 m a.s.l. (Figure 6B), where there are bushes and large patches of *Chusquea* spp., in addition to small ferns, epiphytes and mushroom colonies. We found *P. sira* at night, at the beginning and the end



Figure 6. Habitat in the localities where *Pristimantis sira* sp. nov. occurs: (A) Panoramic view of the Montane forests in El Sira Communal Reserve; (B) forest in Campamento La Cumbre; (C) forest in Campamento Peligroso (type locality). Photographs by Germán Chávez.

Table 1. Range and average ($\pm$ standard deviation) measurements (in mm) of specimens of the type series of *Pristimantis sira* sp. nov. and related cruciform eye’s montane species of the *Pristimantis cruciocularis* group.

	<i>Pristimantis sira</i> sp. nov.		<i>Pristimantis antisuyu</i>		<i>Pristimantis cruciocularis</i>		<i>Pristimantis erythroinguinis</i>	
	Females (n = 4)	Males (n = 1)	Females (n = 6)	Males (n = 2)	Females (n = 7)	Males (n = 7)	Females (n = 2)	Males (n = 4)
SVL	19.0–20.4 (19.7 $\pm$ 0.6)	14.7	17.4–21.1 (20.0 $\pm$ 0.6)	14.3–15.0	18.7–21.8 (20.3 $\pm$ 1.2)	11.4–15.4 (13.5 $\pm$ 1.2)	17.2–17.7	12.2–14.3 (13.0 $\pm$ 0.5)
TL	9.5–10.9 (10.1 $\pm$ 0.6)	8.3	9.8–11.2 (10.8 $\pm$ 0.2)	8.2–8.4	9.6–11.1 (10.5 $\pm$ 0.6)	6.2–8.0 (7.2 $\pm$ 0.6)	9.4–9.5	7.2–8.3 (7.7 $\pm$ 0.2)
FL	8.7–9.2 (9.0 $\pm$ 0.2)	6.9	7.9–9.6 (9.1 $\pm$ 0.3)	6.4–7.0	7.5–8.6 (7.8 $\pm$ 0.5)	4.6–6.5 (5.6 $\pm$ 0.6)	7.4–7.8	5.6–6.5 (6.0 $\pm$ 0.2)
HL	6.3–6.9 (6.7 $\pm$ 0.3)	5.1	6.9–8.2 (7.8 $\pm$ 0.2)	6.1–6.2	6.9–7.9 (7.4 $\pm$ 0.3)	4.6–6.6 (5.2 $\pm$ 0.7)	6.7–7.9	5.2–6.0 (5.7 $\pm$ 0.2)
HW	7.2–7.5 (7.4 $\pm$ 0.1)	5.4	6.4–8.0 (7.2 $\pm$ 0.2)	5.4–5.6	7.1–8.0 (7.6 $\pm$ 0.3)	4.7–5.8 (5.0 $\pm$ 0.4)	5.9–7.0	4.7–5.7 (5.0 $\pm$ 0.2)
ED	2.3–2.8 (2.6 $\pm$ 0.2)	2.0	2.4–2.9 (2.7 $\pm$ 0.1)	2.2–2.3	2.5–2.9 (2.7 $\pm$ 0.1)	1.5–2.4 (1.9 $\pm$ 0.3)	2.5–2.8	2.0–2.1 (2.0 $\pm$ 0)
IOD	1.8–2.0 (1.9 $\pm$ 0.1)	1.7	2.3–2.6 (2.5 $\pm$ 0.1)	1.8–1.9	2.7–2.9 (2.8 $\pm$ 0.1)	1.5–2.2 (1.9 $\pm$ 0.2)	1.8	1.4–1.9 (1.7 $\pm$ 0.1)
EW	1.6–2.0 (1.8 $\pm$ 0.1)	1.6	1.8–2.2 (2.0 $\pm$ 0.1)	1.4–1.8	1.4–2.0 (1.6 $\pm$ 0.2)	1.2–1.5 (1.3 $\pm$ 0.1)	1.8–1.9	1.3–1.7 (1.6 $\pm$ 0.1)
IND	1.7–1.8 (1.8 $\pm$ 0.1)	1.4	1.4–1.9 (1.6 $\pm$ 0.1)	1.3	1.6–2.0 (1.8 $\pm$ 0.1)	1.1–1.4 (1.3 $\pm$ 0.1)	1.3–1.5	0.9–1.2 (1.1 $\pm$ 0.1)
E-N	1.9–2.1 (2.0 $\pm$ 0.1)	1.5	1.9–2.3 (2.2 $\pm$ 0.1)	1.9	1.9–2.4 (2.1 $\pm$ 0.2)	1.4–2.2 (1.7 $\pm$ 0.3)	1.7–2.0	1.3–1.5 (1.4 $\pm$ 0.1)
TL/SVL	0.48–0.57 (0.52 $\pm$ 0.04)	0.56	0.53–0.56 (0.54 $\pm$ 0.01)	0.56–0.57	0.50–0.54 (0.52 $\pm$ 0.02)	0.52–0.55 (0.54 $\pm$ 0.01)	0.53–0.55	0.57–0.61 (0.59 $\pm$ 0.01)
FL/SVL	0.44–0.47 (0.45 $\pm$ 0.01)	0.47	0.44–0.47 (0.45 $\pm$ 0.01)	0.45–0.47	0.37–0.40 (0.38 $\pm$ 0.01)	0.40–0.42 (0.41 $\pm$ 0.01)	0.42–0.45	0.45–0.46 (0.46 $\pm$ 0)
HL/SVL	0.32–0.35 (0.34 $\pm$ 0.01)	0.34	0.37–0.41 (0.39 $\pm$ 0.00)	0.41–0.43	0.33–0.41 (0.37 $\pm$ 0.03)	0.36–0.43 (0.39 $\pm$ 0.03)	0.38–0.46	0.42–0.46 (0.44 $\pm$ 0.01)
HW/SVL	0.37–0.39 (0.38 $\pm$ 0.01)	0.37	0.34–0.38 (0.36 $\pm$ 0.01)	0.36–0.39	0.35–0.40 (0.37 $\pm$ 0.01)	0.35–0.41 (0.37 $\pm$ 0.02)	0.33–0.41	0.37–0.40 (0.38 $\pm$ 0.01)
HW/HL	1.06–1.19 (1.11 $\pm$ 0.05)	1.07	0.87–0.98 (0.93 $\pm$ 0.02)	0.89–0.90	0.97–1.06 (1.03 $\pm$ 0.03)	0.87–1.02 (0.97 $\pm$ 0.07)	0.88–0.89	0.81–0.95 (0.87 $\pm$ 0.03)
E-N/ED	0.70–0.84 (0.79 $\pm$ 0.07)	0.77	0.76–0.82 (0.79 $\pm$ 0.01)	0.83–0.86	0.68–0.85 (0.79 $\pm$ 0.07)	0.74–1.00 (0.88 $\pm$ 0.11)	0.68–0.71	0.62–0.75 (0.67 $\pm$ 0.03)
EW/IOD	0.89–1.00 (0.93 $\pm$ 0.05)	0.97	0.75–0.88 (0.82 $\pm$ 0.02)	0.74–1.00	0.48–0.74 (0.59 $\pm$ 0.10)	0.55–0.80 (0.67 $\pm$ 0.09)	1.00–1.06	0.89–1.00 (0.94 $\pm$ 0.02)

of the rainy season, but could not hear any male calling. Sympatric species were *Pristimantis cruciocularis*, *P. iiap* Padial, Gagliardi-Urrutia, Chaparro & Gutiérrez, 2016, *P. cf. mendax* Duellman, 1978, and *Rhinella nesiototes* Duellman & Toft, 1979. We have noticed illegal mining activity in the foothills of El Sira Communal Reserve. Mining activities could affect the mountain slopes because miners establish camps and build trails to move their equipment across the watersheds, creating disturbance to stream and riverine environments that could cause further deforestation in the type locality of *P. sira*.

Discussion

Previous genetic analyses have shown that a group of *Pristimantis* frogs characterized by cruciform eyes are closely related, and include *P. antisuyu*, *P. cruciocularis*, and *P. erythroinguinis* (Catenazzi and Lehr 2018). Our study suggests that *P. sira* sp. nov. belongs to the same group. Likewise, these species are forming a clade into the larger *P. platydactylus* – *P. llojsintuta* complex (Padial et al. 2009) (see Figure 1). We propose to name the clade “*Pristimantis cruciocularis* group” following Article 61 (Principle of typification) of the International Code of Zoological Nomenclature 1999, which recommends using the oldest bearing-type species belonging within the defined taxonomic boundaries of the group. Additionally, the cruciform eye is a character present in two other species that are not closely related to the *P. cruciocularis* group: *P. ashaninka* and *P. altamazonicus*. *Pristimantis ashaninka* inhabits mountain forests, whereas *P. altamazonicus* is an Amazon lowlands dweller. Our phylogenetic analyses suggest that *P. ashaninka* is not closely related to *P. sira* (but only 16S rRNA fragments are available for *P. ashaninka*). Further analyses and gene sampling are necessary to confirm the relationships of montane species with cruciform eye.

We compared measurements and proportions of *P. antisuyu*, *P. cruciocularis*, and *P. erythroinguinis*, all montane species morphologically similar to *P. sira* (Table 1), concluding that they appear very similar in measurements. We also realised that proportions published in the table 3 of the original description of *P. cruciocularis* (Lehr et al. 2006) seem to be incorrect: males proportions correspond to females and viceversa. Thus, in order to help future research and publish confident morphological data, we present a new measurement dataset of *P. cruciocularis*, including populations formerly assigned to *P. flavobracatus* Lehr, Lundberg, Aguilar & von May, 2006, a junior synonym of *P. cruciocularis* sensu Catenazzi and Lehr (2018).

We have observed illegal gold mining activity near the type locality of *P. sira* sp. nov. Gold mining involves the establishment of mining camps and the subsequent clearing of big areas, which would affect the habitat of the new species. Following the IUCN criteria, and considering the

aforementioned threat and that *P. sira* has been recorded in only two localities with a possible EOO not wider than 6000 km² (which is the entire extension of the El Sira Communal Reserve, SERNANP 2020), we suggest this species to be placed into the Vulnerable category of the IUCN red list.

Acknowledgements

This research would not have been possible without the valuable support of the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) that trusted GC to perform the fieldwork. We are deeply grateful with Servicio Nacional de Áreas Naturales Protegidas (SERNANP) staff who made our work inside El Sira Communal Reserve possible. GC is very pleased to have shared the fieldwork with his friend Jose Malqui, who helped collect the type series of the new species. We also thank Milagros Toala and Katherine Toepfer for their valuable help with fieldwork arrangements.

References

- Aichinger M (1991) A new species of Poison-dart frog (Anura: Dendrobatidae) from the Serranía de Sira, Peru. *Herpetologica* 47: 1–5.
- Barbour T, Dunn ER (1921) Herpetological novelties. *Proceedings of the Biological Society of Washington* 34: 157–162.
- Blackburn DC, Wake DB (2011) Class Amphibia Gray, 1825. In: Zhang Z-Q (Ed.) *Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness*. *Zootaxa* 3148: 38–54. <https://doi.org/10.11646/zootaxa.3148.1.8>
- Boulenger GA (1903) Descriptions of new batrachians in the British Museum. *Annals and Magazine of Natural History* 12: 552–557. <https://doi.org/10.1080/00222930308678892>
- Catenazzi A, Tito A (2016) A new species of *Psychrophrynella* (Amphibia, Anura, Craugastoridae) from the humid montane forests of Cusco, eastern slopes of the Peruvian Andes. *PeerJ* 4: e1807. <https://doi.org/10.7717/peerj.1807>
- Catenazzi A, Lehr E (2018) *Pristimantis antisuyu* sp. n. and *Pristimantis erythroinguinis* sp. n., two new species of terrestrial-breeding frogs (Anura, Strabomantidae) from the eastern slopes of the Andes in Manu National Park, Peru. *Zootaxa* 4394: 185–206. <https://doi.org/10.11646/zootaxa.4394.2.2>
- Catenazzi A, Uscapi V, von May R (2015) A new species of *Noblella* (Amphibia, Anura, Craugastoridae) from the humid montane forests of Cusco, Peru. *Zookeys* 516: 71–84. <https://doi.org/10.3897/zookeys.516.9776>
- Chávez G, Catenazzi A (2016) A new species of frog of the genus *Pristimantis* from Tingo Maria National Park, Huanuco Department, central Peru (Anura, Craugastoridae). *Zookeys* 610: 113–130. <https://doi.org/10.3897/zookeys.610.8507>
- Chávez G, Catenazzi A, Venegas PJ (2017) A new species of arboreal microteiid lizard of the genus *Euspondylus* (Gymnophthalmidae: Cercosaurinae) from the Andean slopes of central Peru with comments on Peruvian *Euspondylus*. *Zootaxa* 4350: 301–316. <https://doi.org/10.11646/zootaxa.4350.2.6>

- Crump ML, Scott Jr NJ (1994) Visual encounter surveys. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS (Eds) Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution, Washington DC, 84–92.
- Duellman WE (1978) Three new species of *Eleutherodactylus* from Amazonian Perú (Amphibia: Anura: Leptodactylidae). *Herpetologica* 34: 264–270.
- Duellman WE, Toft CA (1979) Anurans from Serranía de Sira, Amazonian Perú: Taxonomy and Biogeography. *Herpetologica* 35: 60–70.
- Duellman WE, Lehr E (2009) Terrestrial Breeding Frogs (Strabomantidae) in Peru. Natur und Tier – Verlag GmbH, Germany, 384 pp.
- Duellman WE, Barley AJ, Venegas PJ (2014) Cryptic species diversity in Marsupial Frogs (Anura: Hemiphractidae: *Gastrotheca*) in the Andes of Northern Peru. *Zootaxa* 3768: 159–177. <https://doi.org/10.11646/zootaxa.3768.2.4>
- Echevarria LY, Venegas PJ (2015) A new elusive species of *Petracola* (Squamata: Gymnophthalmidae) from the Utcubamba basin in the Andes of Northern Peru. *Amphibian & Reptile Conservation* 9: 26–33.
- Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS ONE* 2: e1109. <https://doi.org/10.1371/journal.pone.0001109>
- Gagliardi-Urrutia G (2010) Anfíbios y Reptiles de Loreto, Peru. The Field Museum-Rapid Color Guide # 262.
- Hedges SB, Duellman WE, Heinicke MP (2008) New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737: 1–182. <https://doi.org/10.11646/zootaxa.1737.1.1>
- Heinicke MP, Duellman WE, Hedges SB (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academies of Sciences* 104: 10092–10097. <https://doi.org/10.1073/pnas.0611051104>
- Jiménez de la Espada M (1870) Fauna neotropicalis species quaedam nondum cognitae. *Jornal de Ciências, Matemáticas, Physicas e Naturaes*. Lisboa 3: 57–65.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Köhler J, Lötters S (1999) New species of the *Eleutherodactylus unistrigatus* group (Amphibia: Anura: Leptodactylidae) from montane rain forest of Bolivia. *Copeia* 1999: 422–427. <https://doi.org/10.2307/1447487>
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular biology and evolution* 29: 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Lehr E, Moravec J (2017) A new species of *Pristimantis* (Amphibia, Anura, Craugastoridae) from a montane forest of the Pui Pui Protected Forest in central Peru (Región Junín). *Zookeys* 645: 85–102. <https://doi.org/10.3897/zookeys.645.11221>
- Lehr E, von May R (2017) A new species of terrestrial-breeding frog (Amphibia, Craugastoridae, *Pristimantis*) from high elevations of the Pui Pui Protected Forest in central Peru. *Zookeys* 660: 17–42. <https://doi.org/10.3897/zookeys.660.11394>
- Lehr E, Lundberg M, Aguilar C, von May R (2006) New species of *Eleutherodactylus* (Anura: Leptodactylidae) from the eastern Andes of central Peru with comments on central Peruvian *Eleutherodactylus*. *Herpetological monographs* 20: 105–128. [https://doi.org/10.1655/0733-1347\(2007\)20\[105:NSOEAL\]2.0.CO;2](https://doi.org/10.1655/0733-1347(2007)20[105:NSOEAL]2.0.CO;2)
- Lehr E, von May R, Moravec J, Cusi JC (2017a) A new species of *Phrynopus* (Amphibia, Anura, Craugastoridae) from upper montane forests and high Andean grasslands of the Pui Pui Protected Forest in central Peru. *Zookeys* 713: 131–157. <https://doi.org/10.3897/zookeys.713.20776>
- Lehr E, von May R, Moravec J, Cusi JC (2017b) Three new species of *Pristimantis* (Amphibia, Anura, Craugastoridae) from Upper Montane Forests and High Andean Grasslands of the Pui Pui Protected Forest in Central Peru. *Zootaxa* 4299: 301–336. <https://doi.org/10.11646/zootaxa.4299.3.1>
- Lehr E, Moravec J, Cusi JC, Gvozdk V (2017c) A new minute species of *Pristimantis* (Amphibia: Anura: Craugastoridae) with a large head from the Yanachaga-Chemillén National Park in central Peru, with comments on the phylogenetic diversity of *Pristimantis* occurring in the Cordillera Yanachaga. *European Journal of Taxonomy* 325: 1–22. <https://doi.org/10.5852/ejt.2017.325>
- Lötters S, Henzl MJ (2000) A new species of *Atelopus* from the Serranía del Sira, Amazonian Peru. *Journal of Herpetology* 34: 169–173. <https://doi.org/10.2307/1565411>
- Lynch JD (1968) Two new frogs of the genus *Eleutherodactylus* from Eastern Ecuador (Amphibia: Leptodactylidae). *Journal of Herpetology* 2: 129–135. <https://doi.org/10.2307/1563112>
- Lynch JD, Duellman WE (1997) Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in western Ecuador: Systematic, Ecology, and Biogeography. Natural History Museum, The University of Kansas, Lawrence, Kansas, 236 pp. <https://doi.org/10.5962/bhl.title.7951>
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution* 32: 268–274. <https://doi.org/10.1093/molbev/msu300>
- Padial JM, Grant T, Frost DR (2014) Molecular systematics of Terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa* 3825: 1–132. <https://doi.org/10.11646/zootaxa.3825.1.1>
- Padial JM, Gagliardi-Urrutia LAG, Chaparro JC, Gutiérrez RC (2016) A new species of the *Pristimantis conspicillatus* group from the Peruvian Amazon (Anura: Craugastoridae). *Annals of the Carnegie Museum*, Pittsburgh 83: 207–218. <https://doi.org/10.2992/007.083.0302>
- Padial JM, Castroviejo-Fisher S, Köhler J, Vilá C, Chaparro JC, De la Riva I (2009) Deciphering the products of evolution at the species level: the need for an integrative taxonomy. *Zoologica Scripta* 38: 431–447. <https://doi.org/10.1111/j.1463-6409.2008.00381.x>
- Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (2002) Simple fool's guide to PCR: Version 2.0. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, 45 pp.
- Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* 20: 289–290. <https://doi.org/10.1093/bioinformatics/btg412>
- SERNANP (2020) Servicio Nacional de Áreas Naturales Protegidas por el Estado. <https://www.sernanp.gob.pe> [accessed on 30.12.2020]
- Shepack A, von May R, Tito A, Catenazzi A (2016) A new species of *Pristimantis* (Amphibia, Anura, Craugastoridae) from the foothills of the Andes in Manu National Park, southeastern Peru. *Zookeys* 594: 143–164. <https://doi.org/10.3897/zookeys.594.8295>
- Swenson JJ, Young BE, Beck S, Comer P, Córdova JH, Dyson J, Embert D, Encarnación F, Ferreira W, Franke I, Grossman D, Hernandez P,

- Herzog SK, Josse C, Navarro G, Pacheco V, Stein BA, Timaná M, Tovar A, Tovar C, Vargas J, Zambrana-Torrel CM (2012) Plant and animal endemism in the eastern Andean slope: challenges to conservation. *BMC Ecology* 12: 1–1. <https://doi.org/10.1186/1472-6785-12-1>
- von May R, Catenazzi A, Corl A, Santa-Cruz R, Carnaval AC, Moritz C (2017) Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecology and Evolution* 7: 3257–3267. <https://doi.org/10.1002/ece3.2929>
- Vences M, Thomas M, Bonett R, Vieites D (2005) Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society of London B* 360: 1859–1868. <https://doi.org/10.1098/rstb.2005.1717>
- Whitworth A, Beirne C, Pillco Huarcaya R, Serrano Rojas SJ, Chávez G (2016a) Amphibians of the Sira Communal Reserve. The Field Museum-Color Guide # 809.
- Whitworth A, Beirne C, Pillco Huarcaya R, Serrano Rojas SJ, Chávez G (2016b) Reptiles of the Sira Communal Reserve. The Field Museum-Color Guide # 810.

Supplementary material 1

Material examined

Authors: Germán Chávez, Luis A. García-Ayachi, Alessandro Catenazzi

Data type: species data

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/evolsyst.5.63674.suppl1>

Supplementary material 2

Genbank accession codes for specimens considered for phylogenetic analyses.

Authors: Germán Chávez, Luis A. García-Ayachi, Alessandro Catenazzi

Data type: molecular data

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/evolsyst.5.63674.suppl2>

Supplementary material 3

Figure S1

Authors: Germán Chávez, Luis A. García-Ayachi, Alessandro Catenazzi

Data type: image

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/evolsyst.5.63674.suppl3>